REMARKS

Applicants have amended their claims in order to recite that the first base sequence is nonspecific to the base sequence of the target gene, claim 1 being further amended to provide an antecedent basis for the base sequence of the target gene. Moreover, claim 1 has been further amended to delete the recitation, after reciting introduction of the first and second base sequences that such sequences are nonspecific to the base sequence of the target gene.

Applicants respectfully submit that all of the claims presented for consideration by the Examiner patentably distinguish over the teachings of the references applied by the Examiner in rejecting claims in the Office Action mailed July 17, 2006, that is, the teachings of the U.S. patents to Ovyn, et al, No. 6,110,681, and to Livak, et al, No. 5,538,848, United States Patent Application Publication No. US2001/0039014 to Bass, et al, United States Patent Application Publication No. US 2003/0190646 to Wenz, et al., and the articles by Eun, et al, "Simultaneous Quantitation of Two Orchid Viruses, by the TaqMan® Real-Time RT-PCR", in Journal of Virological Methods 87 (2000) 151-160; Leone, et al., "Molecular Beacon Probes Combined With Amplification by NASBA Enable Homogeneous, Real-Time Detection of RNA", in Nucleic Acids Research, 1998, Vol. 26, No. 9, pages 2150-2155, Mackay, et al., "Real-Time PCR in Virology", in Nucleic Acids Research, 2002, Vol. 30, No. 6, pp. 1292-1305, and Rizzo, et al., "Chimeric RNA-DNA Molecular Beacon Assay for Ribonuclease H Activity," in Molecular and Cellular Probes (2002) 16, 227-283, under the provisions of 35 USC 103.

It is respectfully submitted that these references as applied by the Examiner would have neither taught nor would have suggested such a method for expressed gene analysis as in the present claims, having the steps of subjecting the gene to be

analyzed to nucleic acid amplification using, inter alia, (a) the primer for introduction comprising a first base sequence closer to the 5' end of the primer than a third base sequence comprising a sequence specifically hybridizing to a target gene and including a second base sequence closer to the 5' end of the primer than the first base sequence, and (b) the probe comprising a base sequence identical or complementary to the first base sequence, together with steps of digesting the probe and detecting fluorescence, and wherein the gene to be analyzed is prepared by the introduction of the first base sequence which is nonspecific to the base sequence of the target gene and the second base sequence comprising a promoter sequence of RNA polymerase, into the target gene so that the second base sequence is bound to a position closer to a 5' end of the gene to be analyzed than the first base sequence. See claim 1.

As will be shown in the following, it is respectfully submitted that none of the references disclose, or would have suggested, either alone or in combination as applied by the Examiner, preparation of the gene to be analyzed by introduction of a first base sequence which is nonspecific to the base sequence of the target gene and the second base sequence comprising a promoter sequence of RNA polymerase into the target gene in the manner specified in the present claims. Through such introduction and the nonspecific first base sequence and the probe comprising, inter alia, a base sequence identical or complementary to the first base sequence, an advantage is achieved that the probe used in the present invention does not have to be designed for each use in accordance with the base sequence of the target gene and can be universally used regardless of the target gene. The probe used can amplify and detect any type of target gene under substantially the

same conditions and analysis thereof can be simply conducted. See, e.g., pages 28 and 29 of Applicants' specification.

In addition, it is respectfully submitted that the teachings of the references as applied by the Examiner would have neither disclosed nor would have suggested such method for expressed gene analysis as in the present claims, having features as in claim 1 as discussed previously, and, additionally, having features as in the dependent claims, including (but not limited) wherein a gene to be analyzed is cDNA including the first and second base sequences introduced therein, as in claim 2; and/or wherein the nucleic acid amplification is conducted by steps as in claims 3 and 4; and/or wherein the nucleic acid amplification is conducted at a substantially single temperature (see claim 5), in particular where such single temperature is between 37°C and 55°C (see claim 6); and/or wherein the RNA polymerase and the second base sequence are as set forth in claim 7; and/or wherein two or more target genes are simultaneously detected in a single reaction vessel using at least two types of probes (see claim 8), in particular wherein such at least two types of probes have substantially the same melting temperature (see claim 9); and/or wherein the probe is a DNA/RNA hybrid strand (see claim 14).

By use of the primer for introduction as in the present claims, which includes the first, second and third base sequences relatively located to the 5' end of the primer, with the first base sequence being nonspecific to the base sequence of the target gene and the second base sequence comprising a promoter sequence of RNA polymerase, together with the probe comprising a base sequence identical or complementary to the first base sequence, a universal probe for expressed gene analysis which does not have to be designed for each use in accordance with the base sequence of the target gene is achieved. The probe according to the present

invention can amplify and detect any type of target gene under substantially the same conditions, and analysis thereof can be simply conducted. Note, for example, the second paragraph on page 6 of Applicants' specification; see also the paragraph bridging pages 28 and 29 thereof.

Wenz, et al discloses a technique for detection of nucleic acid sequences using coupled ligation and amplification reactions. The most general disclosure of the technique described in Wenz, et al is set forth in paragraph [0007] on page 1 of this patent document. Note also paragraphs [0009] and [0010] on page 1; the Examiner has specifically referred to paragraphs [0028] - [0030} on page 3 of this patent document, and Figs. 1-3 of this patent document.

It is respectfully submitted that Wenz, et al would have neither taught nor would have suggested various aspects of the present invention including wherein the gene to be analyzed is prepared by the introduction of the first base sequence being nonspecific to the base sequence of the target gene and the second base sequence comprising a promoter sequence of RNA polymerase, into the target gene so that the second base sequence is bound to a position closer to the 5' end of the gene to be analyzed than the first base sequence, or use of a probe comprising a base sequence identical or complementary to the first base sequence and labelled at one end with a fluorophore and at another end with a quencher, as in the present claims, and/or other features of the present claims as discussed previously, and advantages thereof.

With respect to claim 1, the Examiner contends that Wenz, et al, "does not teach that the primer for introduction comprises a second base sequence comprising a promoter sequence of RNA polymerase, which is non-specific to the base sequence of the target gene". However, as presently amended, it is respectfully

submitted that Wenz, et al would have neither taught nor would have suggested the first base sequence being nonspecific to the base sequence of the target gene, or the second base sequence comprising a promoter sequence RNA polymerase, or the probe comprising a base sequence identical or complementary to the first base sequence and labelled as recited in the present claims.

It is respectfully submitted that the secondary references as applied by the Examiner in Items 2, 3 and 4 on pages 3-18 of the Office Action mailed July 17, 2006, would not have rectified the deficiencies of Wenz, et al, such that the presently claimed invention as a whole would have been obvious to one of ordinary skill in the art.

Ovyn, et al discloses oligonucleotides that can be used as primers to amplify a region of the 16S rRNA of *Mycoplasma pneumoniae*. Note column 3, lines 39-45 of this patent. Note especially column 7, lines 5-28, of this patent, describing a method for the detection of the specified microorganism. Note also column 3, lines 39-46; column 5, lines 46-67; and column 6, lines 12-16 and 43-61.

It is respectfully submitted that Ovyn, et al would have neither taught nor would have suggested, alone or in combination with the teachings of the other applied references, the presently claimed method, including, inter alia, a first base sequence which is nonspecific to the base sequence of the target gene.

It is respectfully submitted that the upstream and downstream primers in Ovyn, et al only comprise, respectively, a sequence substantially complementary to the target sequence and a sequence substantially homologous to the target sequence. It is respectfully submitted that this patent would have neither taught nor would have suggested, inter alia, a first base sequence nonspecific to the base sequence of the target gene, as in the present claims.

Moreover, it is respectfully submitted that the probe described in Ovyn, et al does not include "a base sequence identical or complementary to the first base sequence". It is respectfully submitted that Ovyn, et al would have neither taught nor would have suggested such probe including such base sequence identical or complementary to the first base sequence, as in the present claims, and advantages thereof as discussed previously.

Livak, et al discloses methods of monitoring the process of nucleic acid amplification reactions, especially polymerase chain reactions. Note, in general, column 3, lines 29-47, for the broadest description of this method. See also column 3, lines 48-55.

Eun, et al discloses simultaneous quantitation of two orchid viruses carried out using the TaqMan® real-time RT-PCR. As for the primer design for the method disclosed in Eun, et al., note Table 1 and the description in Item 2.2 on page 153 of this article.

Even assuming, arguendo, that the teachings of Livak, et al and of Eun, et al were properly combinable with the teachings of Wenz, et al, and Ovyn, et al, such combined teachings would have neither disclosed nor would have suggested the presently claimed subject matter, including, inter alia, the base sequences, including in particular wherein the first base sequence is nonspecific to the base sequence of the target gene, and/or the probe including a base sequence identical or complementary to the first base sequence, and labelled at one end with a fluorophore and at another end with a quencher, and advantages achieved thereby; and/or other features of the present invention as discussed in the foregoing, and advantages thereof.

It is respectfully submitted that the teachings of the references as applied by the Examiner in Items 3 and 4 on pages 9-18 of the Office Action mailed July 17, 2006, would have neither taught nor would have suggested the presently claimed subject matter, including features thereof as discussed previously.

Wenz, et al has been previously discussed.

The article by Leone, et al in Nucleic Acids Research (hereinafter "Leone") discloses employment of molecular beacon probes in a NASBA amplicon detection system to generate a specific fluorescent signal concomitantly with amplification. This article describes the coupling of RNA amplification by NASBA with amplicon detection by molecular beacons technology to produce a homogenous RNA assay, called AmpliDet RNA. Note the first full paragraph in the left-hand column on page 2151 of this article. See also the discussions under the headings "Selection of amplification primers and probe", "Synthesis of the molecular beacons", "NASBA" and "Post-NASBA analysis", on page 2151 of this article.

It is respectfully submitted that Leone, either alone or in combination with teaching of other references as applied by the Examiner, would have neither disclosed nor would have suggested the presently claimed subject matter, including, inter alia, introduction into the target gene of the first base sequence nonspecific to the target gene and specified second base sequence, and the probe, as in the present claims and discussed previously.

The contention by the Examiner on page 10 of the Office Action mailed July 17, 2006, that Leone teaches a primer for introduction including a first base sequence closer to the 5' end than a third base sequence comprising a sequence specifically hybridizing to a target gene and comprising a second base sequence closer to the 5' end than the first base sequence, is noted. However, it is respectfully

submitted that Leone only describes secondary structure models of NASBA amplicons produced by a specified primer set, with each amplicon also containing at its 5' end the transcription initiation sequence from specified primers for the T7 RNA polymerase. It is respectfully submitted that Leone does not describe a primer comprising "a first base sequence" which is nonspecific to the base sequence of the target gene. Thus, it is respectfully submitted that Leone would have neither taught nor would have suggested the primer for introduction as in the present invention, which includes the specified first base sequence.

On page 11 of the Office Action mailed July 17, 2006, the Examiner contends that Leone discloses a probe including a base sequence identical or complementary to the first base sequence and labeled at one end with a fluorophore. To the contrary, it is respectfully submitted that the probe in Leone does not include a base sequence identical or complementary to the first base sequence, and would have neither taught nor would have suggested a probe comprising such base sequence identical or complementary to the first base sequence, as in the present claims, and advantages thereof.

Reference by the Examiner to the article by Leone, et al, in the 1997 Journal of Virological Methods, in the sentence bridging pages 12 and 13 of the Office Action mailed July 17, 2006, is noted. It is emphasized that the Examiner has not set forth this article in the formal statement of the rejection, and has not satisfied requirements of 35 USC 103 (including showing motivation) in connection with this article. If the Examiner intends to maintain reliance on this article, it must be set forth in the formal statement of the rejection and analyzed under requirements of 35 USC 103. See In re Hoch, 166 USPQ 406, 407 n.3 (CCPA 1970).

While it is respectfully submitted that Applicants need not presently address contentions made by the Examiner with respect to the article by Leone, et al. in the 1997 Journal of Virological Methods, the following is noted. Thus, in this article, there is only a description that the sense primers were entirely target specific, whereas the antisense primers consisted of a 3' terminal, target specific sequence and a 5' terminal T7 promoter sequence. See page 21, Section 2.2. It is respectfully submitted that this article does not describe, nor would have suggested, either alone or in combination with the teachings of the other applied references, a primer including a first base sequence which is nonspecific to the base sequence of the target gene. Thus, it is respectfully submitted that the primer described in this article is entirely different from the primer for introduction of the present invention, which includes the specified first base sequence.

Bass, et al discloses automated devices and systems for performing nucleic acid recombination, mutation, shuffling and other diversity generating reactions in vitro. As applied by the Examiner, this publication discloses that as an alternative to TaqMan® is the use of molecular beacons to assess library quality. Note paragraph [0329] on page 36.

Mackay reports on detection of polymerase chain reaction products during real-time. As applied by the Examiner, note, for example, page 1297, right-hand column, of this article.

Even assuming, arguendo, that the teachings of Wenz, et al, Leone, Bass, et al and Mackay, et al were properly combinable, it is respectfully submitted that such combined teachings would have neither disclosed nor would have suggested the presently claimed subject matter, including introduction to the target gene of the first base sequence, nonspecific to the base sequence of the target

gene, and the second base sequence comprising a promoter sequence of RNA polymerase, and other features of the present invention as discussed previously, including the probe with a base sequence identical or complementary to the first base sequence, and advantages thereof.

In connection with the rejection of claim 14 as set forth in Item 4 on pages 15-18 of the Office Action mailed July 17, 2006, the teachings of Wenz, et al and Ovyn, et al, have been previously discussed.

The article by Rizzo, et al discloses preparation of RNA/DNA chimeric molecular beacons, which contain a single-stranded RNA/DNA chimeric oligonucleotide labelled with a 5'-fluorescein as fluorophore and a 3'-DABCYL as quencher, referring to Fig. 1 on page 279 of this article. This article discloses that the fluorophore of the probe is held in proximity to the quencher by the stem-loop structure; and that when the RNA sequence of the RNA:DNA hybrid stem is cleaved, the fluorescence of the fluorophore is manifested. Note the second full paragraph in the left-hand column on page 278 of this article. Note also the paragraph on pages 279 and 280; and the Conclusions set forth in the left-hand column on page 282.

Even assuming, arguendo, that the teachings of Rizzo, et al, were properly combinable with the teachings of Wenz, et al, and of Ovyn, et al, such combined teachings would have neither disclosed nor would have suggested the presently claimed subject matter, including the primer for introduction comprising a first base sequence which is nonspecific to the base sequence of the target gene, and a second base sequence comprising a promoter sequence of RNA polymerase, and with the probe utilized in the method comprising a base sequence identical or complementary to the first base sequence and labelled on one end with a fluorophore and at another end with a quencher.

In view of the foregoing comments and amendments, reconsideration and allowance of all claims presently pending in the above-identified application is respectfully requested.

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Respectfully submitted,

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